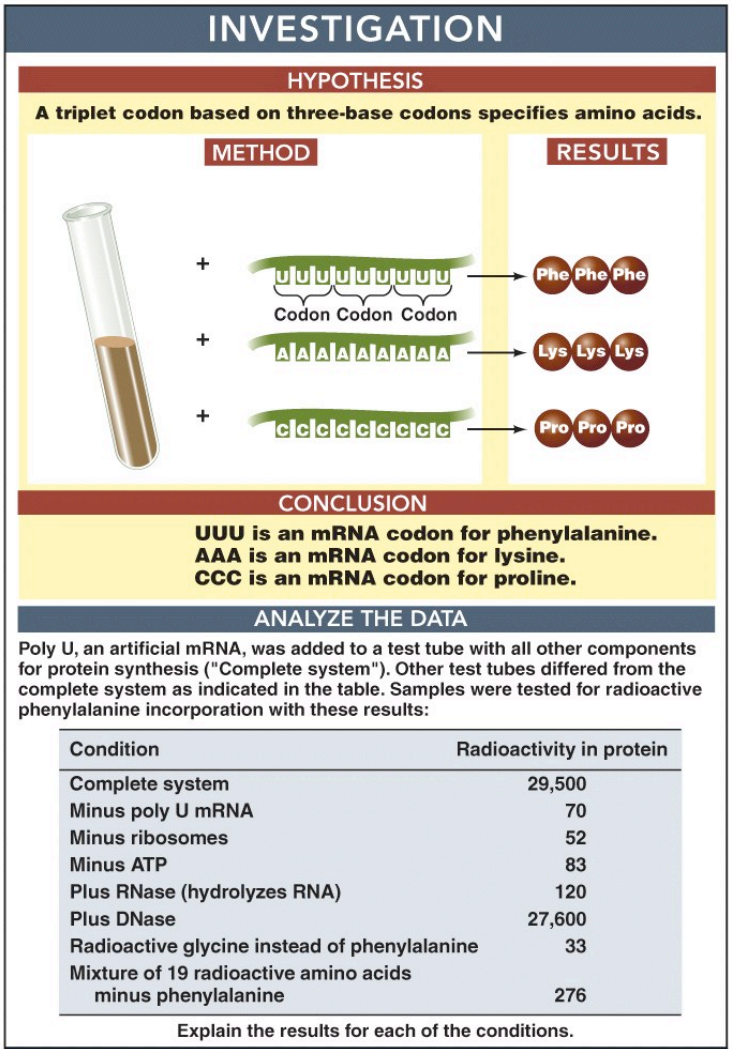
Working With Data from *Principles of Life* by Hillis

Deciphering the Genetic Code

Introduction



By the late 1950s, it was established that DNA was the material, that it replicated semi-conservatively by base pairing, and that it was expressed in proteins. What was not understood was how the nucleotide sequence information in DNA was translated into an amino acid sequence in a protein. Francis Crick proposed that the intermediary between DNA and protein was RNA and genetic evidence pointed to triplets of nucleotides on RNA specifying each amino acid. The race was on to identify which triplet coded for which amino acid. Cell-free systems had been developed, first using rat liver and then using *E. coli*, in which cell extracts were made and protein synthesis occurred. Both systems required ribosomes, ATP and GTP as energy sources, and the cytoplasm that remained after organelles were removed by centrifugation. Protein synthesis was detected by supplying all 20 amino acids, with one of the radioactive. While there was some protein synthesis is this system, it was increased greatly by the addition of extra RNA which could act as mRNA. Different RNAs (e.g., different RNA viruses) primed the synthesis of different proteins, so it was clear that RNA had informational content for genetic coding. Marshall Nirenberg, a scientist at the US National Institutes of Health, tried synthetic RNAs made in the lab in the test tube system and he found that they stimulated protein synthesis. So he and a postdoctoral fellow from Germany, Heinrich Matthaei, got specific: They made a synthetic RNA only with the base uracil (poly U–codon UUU) and tested it in 20 tubes, each one with a different radioactive amino acid. In only one of them, a protein was made, in this case with the amino acid phenylalanine bonded repeatedly to itself. Soon, other codons were identified. In August 1961, Nirenberg and Mattahei submitted a research paper with their results to a journal (see below) and it was published two months later. But before the article was published, Nirenberg, a young, unknown biologist, presented the poly-U experiment data at the International Congress of Biochemistry in Moscow. The audience was sparse, but among the few present was Matthew Meselson, whose work had confirmed the semi-conservative replication of DNA and who immediately realized the importance of this first cracking of the genetic code. Meselson arranged for Nirenberg to present his data the next day at a special session chaired by Francis Crick, and word got around—over 1000 people were present. Nirenberg became an instant scientific celebrity, and received the Nobel Prize in 1968.

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Original Paper

Nirenberg, M. and H. Matthaei. 1961. Dependence of cell-free protein synthesis in *E.coli* upon naturally occurring or synthetic polyribonucleotides. *Proceedings of the National Academy of Sciences* 47:1588–1602.
http://www.pnas.org/content/47/10/1588.full.pdf

Links

(For additional links on this topic, refer to the Chapter 10 Investigation Links.)

Fascinating history of genetic code research, including the Nirenberg-Matthaei experiment
http://history.nih.gov/exhibits/nirenberg/index.htm

Analyze the Data

Question 1

Poly U, an artificial mRNA, was added to a test tube with all other components for protein synthesis ("Complete system"). Other test tubes differed from the complete system as indicated in the table. Samples were tested for radioactive phenylalanine incorporation, with the results in the table below. Explain the results for each of the conditions.

Condition	Radioactivity in protein
Complete system	29,500
Minus poly U mRNA	70
Minus ribosomes	52
Minus ATP	83
Plus RNase (hydrolyzes RNA)	120
Plus DNase	27,600
Radioactive glycine instead of phenylalanine	33
Mixture of 19 radioactive amino acids minus phenylalanine	276

Question 2

Poly U (triangles), or no added RNA (dots), were added to test tubes containing all other components of the protein-synthesizing system. Samples were tested for protein synthesis by radioactive amino acid incorporation after various times, with the following results. What do these data show about the dependence of protein synthesis on added RNA?

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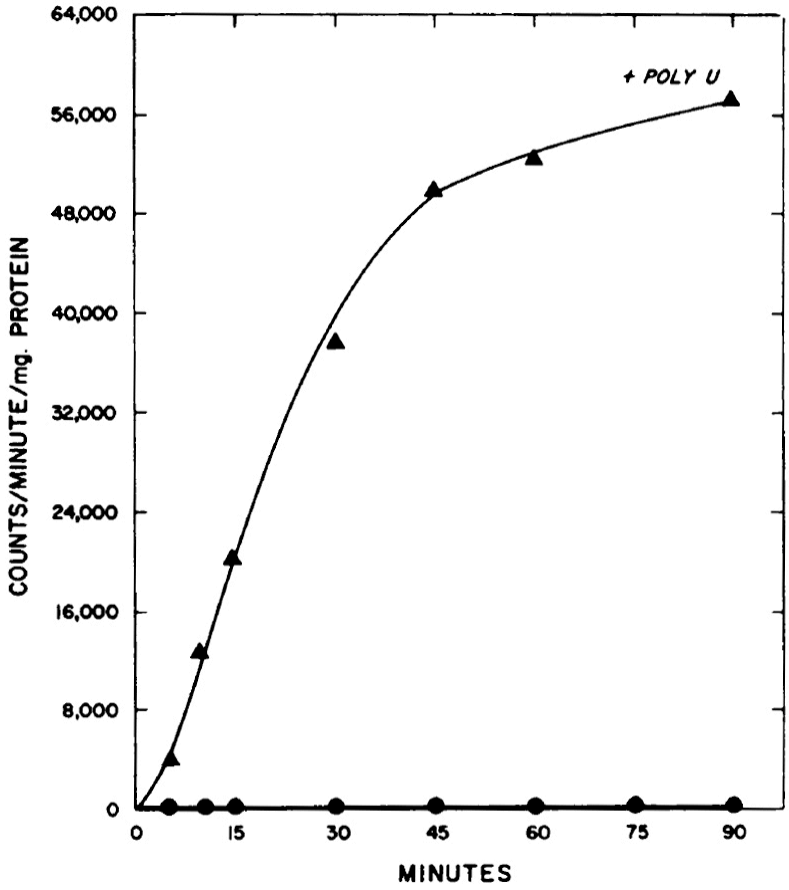

Question 3

The experiment shown in Question 2 above was repeated with different amino acids; the results are in the table below. Explain these results in terms of the codon specificity of poly U.

Radioactive amino acid	Counts/minprotein
Phenylalanine	38,300
Glycine, alanine, serine, aspartic acid, glutamic acid	33
Leucine, isoleucine, threonine, methionine, arginine, histidine, valine, lysine, tyrosine, proline, tryptophan	276
Cysteine	113